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Research Section

Uncertainty factors for chemical risk assessment: interspecies differences in the in vivo pharmacokinetics and metabolism of human CYP1A2 substrates

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Abstract

The 100-fold default uncertainty factor is used to convert a no-observed-adverse-effect level (NOAEL) from a animal toxicity study, to a "safe" value for human intake. The composite uncertainty factor (100) has to allow for interspecies (10-fold) and interindividual (10-fold) differences in toxicokinetics and toxicodynamics. The aim of the current study was to assess the validity of the interspecies default for toxicokinetics (4.0) for each of the test species (dog, rabbit, rat and mouse), using published data for compounds eliminated by CYP1A2 in humans (caffeine, theobromine, theophylline and paraxanthine). An analysis of the published literature showed that the absorption, bioavailability and route of excretion were generally similar between humans and the test species, for each probe substrate. However, interspecies differences in the route of metabolism, and the enzymes involved in this process, were identified. The magnitude of difference in the internal dose, between species, showed that values for the mouse (10.6) and rat (5.4) exceed the 4.0-fold default, whereas the rabbit (2.6) and dog (1.6) were below this value. This work supports the need to replace the generic default factors by a compound-related value derived from specific, relevant, quantitative data; this would result in more relevant and reliable non-cancer risk assessments.

Keywords: Uncertainty factors; Toxicokinetics; Interspecies; CYP1A2; Metabolism

1. Introduction

The prevention of adverse health effects arising from exposure of the human population to compounds that show threshold effects is commonly based on the application of an uncertainty (or safety) factor, to a surrogate threshold value (e.g. a no-observed-adverse-effect level, NOAEL) which has been determined from a long-term, animal toxicity study. In general, the NOAEL in the most sensitive test species (usually determined from a chronic or subchronic feeding study) is divided by an appropriate uncertainty factor to generate an intake value believed to be without "appreciable health risks" to individuals in the human population, if consumed over a lifetime (WHO, 1987); such an exposure value is commonly termed an "acceptable daily intake" (ADI) for food additives and contaminants (Lu, 1985, 1988; Truhaut, 1991). The use of an uncertainty factor in the extrapolation of the NOAEL from an animal study to a human exposure value, allows for the fact that formation and disposition of the proximate toxicant, its dose at the target tissue, and the response of the target site to the toxicant, may vary between humans and the most sensitive test species used for the pivotal toxicity study, and between individuals within the human population. The 100-fold default uncertainty factor was originally proposed, over 40 years ago, by Lehman and Fitzhugh (1954), and still forms the basis of the uncertainty factors which are in use today (Dourson and Stara, 1983; Dourson et al., 1996). The 100-fold factor is considered to be the product of two 10-fold factors; one to move a

Abbreviations: ADI, acceptable daily intake; AUC, area under the plasma concentration–time curve; C_{max}, maximum plasma concentration; IPCS, International Programme on Chemical Safety; NOAEL, no-observed-adverse-effect level; PB-PK, physiologically based-pharmacokinetic.

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dose–response curve in a test species to an exposure value for the average human and the second to move an exposure value in the average human to a value which will cover all of the population including any sensitive sub-groups (WHO, 1987).

This simple and pragmatic approach, adopted in cases where the toxic endpoint is believed to show a threshold, contrasts with the mathematically more sophisticated approaches adopted by some regulatory bodies for carcinogens. As more compound-specific data have become available, the simple linearized low-dose extrapolation of the animal carcinogenicity data has been improved by physiologically-based pharmacokinetic (PB-PK) modelling to estimate the dose delivered to the target organ, and in some cases even a full biologically-based, dose–response model in which the low-dose extrapolation takes into account species differences in both PB-PK and target organ response. For toxicants that show a threshold effect, a more flexible regulatory framework that will permit the incorporation of scientific, biologically-based, data has been proposed recently, in which each 10-fold uncertainty factor is subdivided into two further subfactors, for toxicokinetics (the relationship between the external dose and the internal dose) and toxicodynamics (the relationship between the internal dose and the effect) (Renwick, 1993). This framework permits the replacement of any of the four subfactors if compound-specific data are available (Renwick, 1993), and the product of the compound-related values with remaining default factors would give a "data-derived uncertainty factor". The International Programme on Chemical Safety (IPCS) workshop on the derivation of guideline values (WHO, 1994) supported the subdivision of the 10-fold factors with the human variability factor given equal weighting for toxicokinetics and toxicodynamics (Fig. 1). The recent analysis of Renwick and Lazarus (1998) is consistent with the subdivision shown in Fig. 1.

The approaches used in non-cancer risk assessment currently range from the application of a default uncertainty factor of 100 to the NOAEL from animal studies, to a full biologically-based, dose–response model. However, the procedure that is adopted is dependent on the extent of relevant, quantitative, compound-specific data, which allow the simplistic approaches to be replaced by an extrapolation which uses available compound-specific data in a quantitative manner. However, for the majority of chemicals, there is an absence of data that can support the underlying biological processes involved in determining the internal dose of a chemical (toxicokinetics) or the interaction of a chemical with the target site (toxicodynamics). In many cases the metabolic fate of the chemical may be known, or could be determined easily, in animals and/or humans from in vivo and/or in vitro studies. Although such simple metabolic data currently contribute qualitatively to the

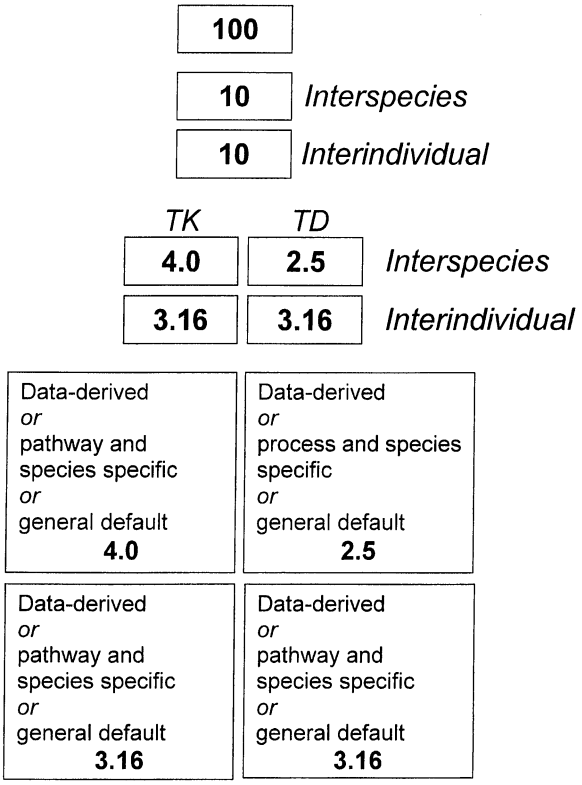

Fig. 1. The subdivision of the 100-fold default uncertainty factor (Renwick and Lazarus, 1998).

risk assessment process they have not been used in a quantitative manner. The development of uncertainty factors for different metabolic fates in the different test species would allow the usual default of 100-fold to be refined to a "species- and pathway-related default" (Fig. 1). Such a default could then be applied on a categorical[1] basis to compounds for which metabolic but not kinetic data are available. The current project is part of a larger programme of research to evaluate the potential use of "categorical defaults", using previously published pharmacokinetic data in order to reduce the uncertainties used in making interspecies comparisons.

The necessity for an interspecies uncertainty factor is based on the principle that an average group of humans may be 10-fold more susceptible to a toxicant than the average group of animals in which the NOAEL has been determined (WHO, 1987; Renwick, 1991). In terms of toxicokinetics, the area under the plasma concentration–time curve (AUC) essentially describes the internal exposure of an animal to a toxicant, which is in turn is a powerful predictor of toxicity during chronic treatment. For most toxicants, the larger and more prolonged the exposure, the greater the toxicity and hence the lower the NOAEL. The ratio of the clearance of toxicant from

[1] Related to Categories (Webster's Dictionary); equivalent to categorical (Oxford Dictionary).

the body (determined by the dose administered divided by the AUC) in animals, to the same parameter in humans, will provide an estimate of the extent to which this difference is likely to lead to the animal species underestimating the likely human internal exposure (Renwick, 1993). For acute toxicity, the relevant interspecies comparison could be based on either AUC (or clearance) or maximum concentration (C_{max}). PB-PK models have an advantage over simple physiologically-based, kinetic parameters, such as clearance, in their ability to model the peak concentration in the target organ; the AUC in the target compared to the AUC in plasma will be reflected by the apparent volume of distribution (which does not usually show important species differences on a body weight basis). An advantage of using in vivo physiologically based parameters (such as clearance and bioavailability) is that all in vivo processes are included in the estimate, including the contribution of extrahepatic tissues to total clearance.

The application of the same uncertainty factor (100-fold) to data for different test species, and to compounds with different metabolic fates is difficult to justify on scientific grounds. The uncertainty factor is applied to an external dose, expressed on a mg/kg body weight basis, irrespective of the test species showing the critical effect in the pivotal toxicity study (Lu, 1985, 1988; Truhaut, 1991). However, the basic physiological processes (such as liver weight and liver blood flow) vary between the common laboratory species and humans (Table 1). This is especially true for the mouse where there are large differences in liver weight, organ blood flow and surface area as compared to humans (Calabrese et al., 1992; Table 1). The scaling of kinetic parameters between species could be made on the basis of body surface area rather than body weight. Surface area can be scaled by the (body weight)$^{0.66}$ while intermediary metabolism is related to (body weight)$^{0.75}$ (Kleiber et al., 1956). Such scaling is appropriate for physiological functions and intermediary metabolism, but is of less relevance for xenobiotic metabolising enzymes.

Table 1
Average values of liver weights and hepatic blood flows in humans and animals (Boxenbaum, 1980)

Species	Body weight (kg)	Liver weight (% of body weight)	Hepatic blood flow ml/min/kg
Human	63–70	2.4	28–25
Dog	17	2.9	50
Rabbit	2.8 2.9	4.8	42
Rat	0.22–0.25	4.0	69–77
Mouse	0.0304	5.0	86

The hepatic oxidative-biotransformation of foreign compounds is mediated mainly by enzymes of the cytochrome P450 (EC 1.14.14.10) superfamily (Guengerich, 1988). Research over the past decade has demonstrated that there are marked species differences in both the total amounts of these enzymes (Table 2) and specific isoforms present, with more than 30 different forms in each species (Nelson et al., 1991; Table 2). The CYP1A2 isoform has been studied extensively, and shows a high degree of amino acid sequence homology, and substrate specificity, between humans and the major test species (Kawajiri and Hayashi, 1996).

The aim of the current paper is to establish "CYP1A2 related defaults" for interspecies differences in toxicokinetics, using probe compounds for which pharmacokinetic data are available in the scientific literature, and for which the major route of elimination is oxidative metabolism, catalysed by CYP1A2 in humans. This analysis will permit the development of interspecies toxicokinetic uncertainty factors which are related to the oxidative metabolism by CYP1A2 in humans. The current analysis combines published pharmacokinetic

Table 2
Major cytochrome P450 enzymes in humans and the four test species

Human[a]		Dog[bcd]		Rabbit		Rat[e]		Mouse	
Total P450 (nmol/mg protein)	231[f]	685[f]		681[f]		444[f]		719[f]	
P450	% of total	P450	% of total	P450	% of total	P450	% of total	P450	% of total
1A2	13	1A1/2	3	1A2	?	1A2	2	1A2	?
2A6	4			2A4	?	2A1	7	2A	?
2B6	0.2	2B11	11	2B	?	2B1/2	5	2B	?
2C	20	2C21	38	2C	?	2C11	54		
2D6	~2	2D16[e]	20			2D1			
2E1	7			2E	?	2E1		2E1	?
3A4	30	3A12	18	3A6	?	3A1	17		

[a] Shimada et al. (1994).
[b] Eguchi et al. (1996).
[c] Sakamoto et al. (1995).
[d] Shiraga et al. (1994).
[e] Nedlecheva and Gut (1994).
[f] Tanaka et al. (1999).

information with metabolism data in the human, dog, rabbit, rat and mouse. Only by combining pharmacokinetic and metabolic data can species similarities and differences be adequately assessed, and the use of "categorical defaults" for toxicokinetics be developed.

2. Methods

Literature searches were undertaken using MEDLINE (1966–1999), BIDS-EMBASE (1980–1999) and TOXLINE (1966–1999), employing search terms initially aimed at identifying probe substrates for human CYP1A2. The compounds identified in this primary screen were selected on the basis that they met the following criteria in humans:

1. complete absorption from the gastrointestinal tract after oral administration, and
2. at least 60% of the compound is metabolised in vivo in humans by CYP1A2.

Published pharmacokinetic and metabolism studies for the compounds that fulfilled the above criteria were searched using the databases, employing the following search terms:

1. compound and pharmacokinetics,
2. compound and metabolism, and
3. compound and CYP1A2.

The abstracts describing human and/or animal studies were screened for metabolism and/or pharmacokinetic data, and full copies of publications that contained useful information or data were evaluated for "quality" (dosing regimen, time range for AUC, accuracy of analytical method and dose-dependent pharmacokinetics). The total clearance value adjusted to body weight (ml/min/kg) and C_{max}/dose [(ng/ml)/(mg/kg)] after oral administration were the pharmacokinetic parameters used as a measure of the internal dose for interspecies comparisons. However, if the published values for the animals studies were reported as clearance (ml/min) or area under the plasma concentration curve (AUC), then these values were converted to clearance (ml/min/kg) by dividing the published mean clearance (ml/min) value by the published mean weight of the animal (kg), or the mean dose (mg/kg) administered by the mean AUC. The primary parameter used, clearance (ml/min/kg) was that determined after oral administration (clearance/F, where F = bioavailability), since this route is used for chronic toxicity testing and both clearance and bioavailability can affect the internal dose. Clearance data following intravenous administration (ml/min/kg) were also analysed, because a significant proportion of the animal studies in the literature were reported as using this route of exposure. When used, these values were compared wherever possible to data from studies in humans given intravenous doses. Differences in mean clearance estimates would represent both inter-species differences plus measurement errors; clearance values are derived from multiple measurements, so that random analytical errors would not greatly influence the value, while systematic errors would be minimised by the use of data from a number of different studies, especially for the mean human data.

The pharmacokinetic values from the published studies were extracted and the mean pharmacokinetic parameter and standard deviation for each compound weighted according to the number of individuals in the study. The human studies used were all for non-smoking, healthy, adults. For the evaluation of metabolism studies, all of the percentages quoted in the tables are given as the percentage of that compound excreted in the urine, rather than the percentage of the total dose.

3. Results

3.1. Caffeine

3.1.1. Human

Caffeine (1,3,7-trimethylxanthine) is present in more than 60 plant species throughout the world, and in beverages such as coffee and tea. It is also used therapeutically as a bronchodilator (Dollery, 1998). The absorption of caffeine from the gastrointestinal tract is rapid and complete with a bioavailability that is close to unity (Blanchard and Sawers, 1983). The majority of a dose is excreted in the urine in the form of metabolites (70–89%) with little unchanged (Table 3). There are four primary routes of metabolism in humans: 1-, 3- and 7-*N*-demethylation and C-8-oxidation (Latini et al., 1981; Ferrero and Neims, 1983). The formation of 1,7-dimethylxanthine (paraxanthine), 3,7-dimethylxanthine (theobromine) and 1,3-dimethylxanthine (theophylline) account for 80, 11 and 4% of the primary *N*-demethylations, respectively (Lelo et al., 1986a). The C-8-oxidation of caffeine is only a minor route of elimination in humans (Table 3). Each primary metabolite is further biotransformed, either by *N*-demethylation or C-8-oxidation, and the metabolites excreted in the urine mostly represent these secondary reactions (Table 3).

High- and low-affinity enzymes catalyse the primary *N*-demethylation, and C-8-oxidation, reactions of caffeine in human hepatic microsomes, with the high-affinity enzyme predominating at low (in vivo) concentrations (Campbell et al., 1987). Several studies have now identified the high-affinity enzyme as CYP1A2, whereas the low-affinity components of caffeine 1- and 7-*N*-demethylations correlate with levels of CYP2E1 in the liver (Campbell et al., 1987; Gu et al., 1992; Tassaneeyakul et al., 1994; Ha et al., 1996; Rasmussen et al., 1998). The C-8-oxidation of caffeine, a minor route of elimination,

is catalysed by a number of isoforms in vitro (CYP2E1, CYP3A4 and CYP3A5), although CYP3A4 is likely to be mostly involved in this reaction in vivo (Aldridge et al., 1979; Cazeneuve et al., 1994; Tassaneeyakul et al., 1994; Rasmussen et al., 1998). At plasma concentrations in the range of 20 to 40 μmol/l, the pharmacokinetic clearance of caffeine is a good probe to assess the in vivo activity of human CYP1A2 (Ha et al., 1996).

Non-linear kinetics have been observed at doses above 10 mg/kg in humans (Bonati and Garrattini, 1984), and studies that exceeded this were excluded from the current database (Table 3). The mean total clearance for caffeine, was 1.2 and 1.9 ml/min/kg after oral and intravenous dosing in 163 and 20 healthy adults, respectively (Table 7) [see Dorne et al. (2001) for references].

3.1.2. Dog

Following the intravenous administration of caffeine to adult dogs, the majority (60–92%) of the dose is excreted in the urine (Aldridge and Neims, 1979; Warzawski et al., 1982) as metabolites (Table 3). Aldridge and Neims (1979) suggested that 7- and/or 3-*N*-demethylation of caffeine are the primary route(s) of metabolism and urinary metabolite data in the beagle dog (at 22 days of age) showed that the major route of caffeine biotransformation is 7-*N*-demethylation (Warzawski et al., 1982; Table 3).

The 7-*N*-demethylation of caffeine in the dog is catalysed by phenobarbital-inducible cytochrome P450s (CYP2B11, 2C11 and 3A12; Eguchi et al., 1996), whereas 1-*N*- and 3-*N*-demethylation, and C-8-oxidation are catalysed by the inducible CYP1A subfamily (Aldridge and Neims, 1979, 1980; Warszawski et al., 1982). There were no data in the literature that described the dose at which non-linear kinetics occurs in the dog, and the doses used in the current analysis ranged from 2 to 50 mg/kg. An analysis of the published data showed that the pharmacokinetic clearance of caffeine, after intravenous administration, in 13 dogs was 2.3 ml/min/kg (Gorodischer et al., 1977; Aldridge and Neims, 1979; Boothe et al., 1994; Tanaka et al., 1998), 1.2-fold higher than in humans (Table 7).

Table 3
Urinary metabolites of caffeine in humans and animals[ab]

Pathway of metabolism	Species				
	Human[c]	Dog[d]	Rabbit[e]	Rat[f]	Mouse[g]
Primary *N*-demethylations			1,3-DMX (2)	1,3-DMX (10)	1,3-DMX (1–3)
	1,7-DMX (5–10)	1,7-DMX (2)	1,7-DMX (12)	1,7-DMX (22)	1,7-DMX (14–17)
	3,7-DMX (2 3)	3,7-DMX (3)	3,7-DMX (4)		
	1,3-DMU (7–11)	1,3-DMU (6)	1,3-DMU (2)	1,3-DMU (6)	
	1,7-DMU(3–11)		1,7-DMU (3)	1,7-DMU (5)	1,7-DMU (7)
			3,7-DMU (3)		
	1-MX (15–16)	1-MX (2)	1-MX (19)	1-MX (7)	1-MX (7)
	3-MX (3–4)	3-MX (30)	3-MX (3)		
	7-MX (6–10)		7-MX (13)		7-MX (7)
	1-MU (31–51)	1-MU (22)	1-MU (16)	1-MU (8)	1-MU (10–11)
			3-MU (4)		
		7-MU (1)			
	AFMU (0–2)				
C-8-oxidation	1,3,7-TMU (1–4)	1,3,7-TMU (3)	1,3,7-TMU (2)	1,3,7-TMU (12)	1,3,7-TMU (6)
			3,6,8-TMA (0.4)	3,6,8-TMA (6)	
	ADMU (0–1)		ADMU (4)	ADMU (27)	ADMU (12)
Unchanged	< 6	1	0.4	< 5	1–6

[a] Values in parentheses are the percentage of the metabolite excreted in the urine.

[b] Abbreviations: 1,3,7-TMX = 1,3,7-trimethylxanthine (caffeine); 1,3-DMX = 1,3,-dimethylxanthine (theophylline); 3,7-DMX = 3,7-dimethylxanthine (theobromine); 1,7-DMX = 1,7-dimethylxanthine (paraxanthine); 1,3-DMU = 1,3-dimethyluric acid; 1,7-DMU = 1,7-dimethyluric acid; 3,7-DMU = 3,7-dimethyluric acid; 1-MX = 1-methylxanthine; 3-MX = 3-methylxanthine; 7-MX = 7-methylxanthine; 1-MU = 1-methyluric acid; 3-MU = 3-methyluric acid; 7-MU = 7-methyluric acid; UA = uric acid; AMMU = 6-amino-5[*N*-methylformylamino]-1-methyluracil; 1,3,7-TMU = 1,3,7-trimethyluric acid; AFMU = 5-acetylamino-6-formyl-amino-3-methyluracil; ADMU = 4-amino-5(5-formylmethylamino)-1,3-dimethyluracil; 3,6,8-TMA = 3,6,8-trimethylallantoin.

[c] Bonati et al. (1982); Tang et al. (1983); Latini et al. (1981).

[d] Aldridge and Neims (1980).

[e] Beach et al. (1983).

[f] Latini et al. (1981).

[g] Arnaud (1985); Burg and Stein (1972).

3.1.3. Rabbit

The absorption of caffeine from the gastrointestinal tract of the rabbit is extensive, with no significant first-pass metabolism (Beach et al., 1983; Bonati et al., 1984b; Dorrbecker et al., 1987). The predominant route of excretion is the urine (82%), of which only a small proportion is unchanged (Table 3). Bonati and Garattini (1984) reported that 3-*N*-demethylation is the predominant elimination pathway and the urinary metabolites are mostly derived from the further metabolism of 1,7-dimethylxanthine (Table 3). The CYP1A2 isoform is the dominant enzyme catalysing the formation of 1,7-dimethylxanthine in rabbit liver (Berthou et al., 1992).

The elimination of caffeine is saturable at doses exceeding 10 mg/kg (Bonati et al., 1984b) and the doses used for the current analyses did not exceed this value. The mean pharmacokinetic clearance following oral administration was 5.9 ml/min/kg (Bonati et al., 1984a), which is 4.9-fold faster than in humans (Table 7). After intravenous administration the mean pharmacokinetic clearance from 22 rabbits was 5.0 ml/min/kg (Beach et al., 1983; Dorrbecker et al., 1987; McNamara et al., 1992), 2.6-times greater than the same parameter in humans (Table 7).

3.1.4. Rat

The majority of an oral dose of caffeine is absorbed from the gastrointestinal tract of the rat (F = 90%) and excreted in the urine (57–68%) (Arnaud, 1976, 1985; Welch et al., 1977) as metabolites (Table 3). The rat metabolises caffeine by 1-, 3- and 7-*N*-demethylation (Welch et al., 1977), and extensively by C-8-oxidation (Table 3). The CYP1A2 isoform can catalyse caffeine *N*-demethylation in the rat (Welch et al., 1977; Berthou et al., 1992; Fuhr et al., 1992; Bienvenu et al., 1993; Chung et al., 1998). However, the C-8-oxidation of caffeine, the important route of elimination in vivo, is catalysed by CYP1A2, 2B1, 2E1 and 3A1 (Fuhr et al., 1992; Bienvenu et al., 1993; Chung et al., 1998).

Saturable pharmacokinetics occur at doses exceeding 10 mg/kg (Bonati et al., 1984b) and the studies used for the current analysis ranged from 1 to 10 mg/kg. The mean pharmacokinetic clearance in 41 rats after oral dosing was 12.0 ml/min/kg (Latini et al., 1980; Bonati et al., 1984b), 10.0-fold more rapid than in humans (Table 7). After intravenous dosing to 21 rats the mean pharmacokinetic clearance was 4.6 ml/min/kg (Aldridge et al., 1977; Ismahan Okyayuz et al., 1985; Tanaka et al., 1994), which is 2.4-fold greater than the same parameter in humans (Table 7).

3.1.5. Mouse

The majority of an oral dose of caffeine is absorbed from the gastrointestinal tract of the mouse and excreted in the urine (70–80%) as metabolites (Table 3). The 1-*N*-demethylation of [1-Me-^{14}C]caffeine accounts for around 14% of the primary metabolism of caffeine (Arnaud, 1985). A considerable amount of caffeine also undergoes C-8-oxidation (Table 3). A comparison of the in vivo pharmacokinetics and metabolism of caffeine in wild-type mice and those lacking CYP1A2 (−/−) demonstrated that 87% of the caffeine clearance is mediated by CYP1A2 (Buters et al., 1996), which is supported by an in vitro study using cell lines expressing the isolated enzyme (Fuhr et al., 1992).

The pharmacokinetics of caffeine are linear up to a dose of 100 mg/kg dose in the mouse (Bonati et al., 1984b). After an intravenous dose of 1 or 2.5 mg/kg the mean pharmacokinetic clearance of caffeine was 12.2 ml/min/kg (Bonati et al., 1984b), 10.2-fold higher than in humans (Table 7).

Table 4
Urinary metabolites of paraxanthine in humans[a]

Pathway of metabolism	Species
	Human[b]
1-*N*-demethylation	7-MX (6)
7-*N*-demethylation	1-MX (20) 1-MU (39) AFMU (16)
C-8-oxidation	1,7-DMU (9)
Unchanged	10

[a] Lelo et al. (1986b).
[b] For abbreviations, see Table 3.

Table 5
Urinary metabolites of theobromine in humans and animals[a]

	Species		
Pathway of metabolism	Human[b]	Rabbit[c]	Rat[d]
3-*N*-demethylation	7-MX (39–49) 7-MU (11–13)	7-MX (42–56) 7-MU (2–6)	7-MX (3–4) 7-MU (2–3)
7-*N*-demethylation	3-MU (18–24)	3-MX (10–13) 3-MU (1–5)	3-MX (6) 3-MU (< 0.2)
C-8-oxidation	AMMU (8–14) 3,7-DMU (2)	AMMU (12–15) 3,7-DMU (2–3)	AMMU (25–42) 3,7-DMU (3–12)
Unchanged	11–23	7–14	45–58

[a] For abbreviations, see Table 3.
[b] Birkett et al. (1985); Miners et al. (1982); Rao et al. (1973); Rodopolous et al. (1996); Shively et al. (1985); Tarka et al. (1983).
[c] Miller et al. (1984); Traina and Bonati (1985).
[d] Miller et al. (1984); Shively et al. (1986); Bonati et al. (1984a); Shively and Vesell (1987).

3.2. Paraxanthine

3.2.1. Human

The absorption of paraxanthine (1,7-dimethylxanthine) from the gastrointestinal tract is extensive, with the majority (~90%) excreted in the urine as metabolites formed after 7-*N*-demethylation (Table 4). Both primary demethylation reactions of paraxanthine are exclusively catalysed by CYP1A2 in human hepatic microsomes (Campbell et al., 1987) and cell lines (Gu et al., 1992). The C-8-oxidation of paraxanthine is catalysed by CYP1A2 and CYP2A6, at similar rates in vitro (Gu et al., 1992) but the former enzyme is more abundant in human liver and its contribution is likely to be dominant in vivo (Table 2). As the 1-*N*- and 7-*N*-demethylation pathways predominate in vivo paraxanthine pharmacokinetics can be used as probe to study CYP1A2 activity in vivo.

The dose used in the single, published study was approximately 4 mg/kg and the mean clearance after an oral dose in six healthy adults was 1.7 ml/min/kg (Table 7; see Dorne et al., 2001 for references).

3.2.2. Rat

There are no published data describing the absorption or metabolism of paraxanthine in the rat. However,

Table 6
Urinary metabolites of theophylline in humans and animals[a]

	Species				
Pathway of metabolism	Human[b]	Dog[c]	Rabbit[c]	Rat[d]	Mouse[e]
1-*N*-demethylation	3-MX (14–17)	3-MX (40)	3-MX (1) 3-MU (0.3)	–	–
3-*N*-demethylation	1-MU (17–24)	–	1-MX (1) 1-MU (2–6)	1-MU (7–20) UA (1)	1-MX (40)
C-8-oxidation	1,3-DMU (39–48)	1,3-DMU (27–40)	1,3-DMU (75–78)	1,3-DMU (20–30)	1,3-DMU (40)
Unchanged	8–17	20–30	9–15	30	15

[a] For abbreviations, see Table 3.
[b] Birkett et al. (1985); Jenne et al. (1976); St-Pierre et al. (1985).
[c] Rackley et al. (1991); Saunier et al. (1987).
[d] Bouraoui et al. (1995); Celardo et al. (1985).
[e] Teunissen (1985).

Table 7
Mean pharmacokinetic values and ratios for substrates of CYP1A2 in humans and animals[a]

Compound, parameter and route of administration	Human	Dog	Rabbit	Rat	Mouse
Caffeine					
Clearance (oral)	1.2 (163)[11]	–	5.9 (ns)[1]	12.0 (41)[2]	12.2 (3)[1]
			4.9	**10.0**	**10.2**
Clearance (IV)	1.9 (20)[2]	2.3 (13)[4]	5.0 (22)[3]	4.6 (21)[3]	–
		1.2	**2.6**	**2.4**	
Paraxanthine					
Clearance (oral)	1.7 (6)[1]	–	–	16.1 (20)[1]	–
				9.5	
Theobromine					
Clearance (oral)	1.0 (45)[6]	–	1.8 (32)[1]	3.1 (39)[3]	–
			1.8	**3.1**	
Theophylline					
Clearance (oral)	0.9 (106)[6]	2.2 (4)[1]	2.4 (18)[1]	1.9 (62)[1]	–
		2.4	**2.7**	**2.1**	
Clearance (IV)	1.0 (100)[11]	1.5 (25)[5]	1.5 (63)[5]	3.0 (65)[8]	11.0 (3-4)[1]
		1.5	**1.5**	**3.0**	**11.0**
C_{max}/dose (oral)	4598.7 (32)[3]	–	1311.0 (18)[1]	–	–
			3.5		
Mean ratio					
Clearance (oral)		**2.4**	**3.1**	**6.2**	**10.2**
Clearance (IV)		**1.4**	**2.1**	**2.7**	**11.0**
Clearance (IV and oral)		**1.6**	**2.6**	**5.4**	**10.6**
Mean ratio					
C_{max}/dose			**3.5**		

[a] (Number) = total number of determinations which contributed to the mean pharmacokinetic parameter and [x] = number of publications. The number in bold type is the ratio of animal clearance/human clearance or human (C_{max}/dose)/animal C_{max}/dose) where, clearance = ml/min/kg and C_{max}/dose = (ng/ml)/(mg/kg). (ns) = not stated.

after a single, oral dose the mean pharmacokinetic clearance in 20 rats was 16.1 ml/min/kg (Bortolotti et al., 1985), 9.4-fold greater than the same parameter in humans.

3.3. Theobromine

3.3.1. Human

Theobromine (3,7-dimethylxanthine) is a natural constituent of cocoa, a minor component in tea and coffee and a primary metabolite of caffeine. After oral administration it is completely absorbed from the gastrointestinal tract, and excreted in the urine (86–99%) as metabolites (Table 5). The three primary routes of theobromine metabolism in humans are 3- and 7-*N*-demethylation, and C-8-oxidation (Table 5). Theobromine 3-*N*-demethylation is catalysed exclusively by CYP1A2, whereas the 7-*N*-demethylation is catalysed by CYP1A2, 2A6 and 2E1 with 2E1 the most efficient isoform (Campbell et al., 1987; Gu et al., 1992; Gates and Miners, 1999). The C-8-oxidation reaction is catalysed at similar rates by both CYP1A2 and CYP2E1 in vitro (Gu et al., 1992; Gates and Miner, 1999), although the former is likely to predominate in vivo because it is at higher levels in the human liver (Table 2). The data available demonstrate that CYP1A2 makes an extensive contribution to the overall elimination of theobromine and as such the pharmacokinetic clearance of theobromine can used to estimate human CYP1A2 activity in vivo.

The pharmacokinetics studies used in the current analysis administered doses ranging from 0.4 to 10 mg/kg. The mean pharmacokinetic clearance was 1.0 ml/min/kg, after an oral dose to 45 healthy adults (Table 7; see Dorne et al., 2001 for references).

3.3.2. Rabbit

The absorption of theobromine after an oral dose in the rabbit is complete, with high bioavailability (~100%). After oral administration, most (80–85%) of a dose is excreted in the urine (Latini et al., 1984; Miller et al., 1984), as metabolites (Table 5). The major metabolites are derived from an initial 3-*N*-demethylation of theobromine (Table 5). No published data are available that describe the oxidative enzyme(s) which catalyse the metabolism of theobromine in the rabbit.

There is no evidence of saturable pharmacokinetics at doses up to 100 mg/kg (Latini et al., 1984). The mean pharmacokinetic clearance from 32 rabbits was 1.8 ml/min/kg (Latini et al., 1984), 1.8-times greater than in humans (Table 7).

3.3.3. Rat

Theobromine is well absorbed from the gastrointestinal tract of the rat, having bioavailability which is close to unity (Shively and Tarka, 1983; Bonati et al., 1984a), but with the main elimination pathways differing quantitatively from those in humans. The majority (66–100%) of an oral dose is excreted in the urine (Arnaud and Welsch, 1979), of which a large proportion is unchanged (Table 5). The major urinary metabolites of theobromine are generated after C-8-oxidation with both the primary *N*-demethylation reactions relatively minor routes of elimination (Table 5).

Pretreatment of rats with 3-methylcholanthrene (CYP1A inducer) significantly increases the plasma clearance of theobromine, and the urinary excretion of the C-8-oxidative metabolite, 6-amino-5[*N*-methylformylamino]-1-methyluracil (Shively and Vesell, 1987). However, antibodies to 3-methylcholanthrene-induced microsomes have no effect on the hepatic metabolism of theobromine in vitro using liver from non-induced animals (Shively and Vesell, 1987). It cannot be concluded that a member of the CYP1A is responsible for the metabolism of theobromine in vivo in the control rat.

The pharmacokinetics of theobromine are linear at doses ranging from 1 to 100 mg/kg (Bonati et al., 1984a). The mean pharmacokinetic clearance of theobromine in 39 rats was 3.1 ml/min/kg (Shively and Tarka, 1983; Bonati et al., 1984a; Shively and Vesell, 1987; Table 3), 3.1-times higher than the same parameter in humans (Table 7).

3.4. Theophylline

3.4.1. Human

Theophylline (1,3-dimethylxanthine) is a naturally occurring alkaloid found in tea and also used medically as a bronchodilator, as well as in the prevention of apnea of premature and older infants (Dollery, 1998). After administration by oral or intravenous routes the majority (92–100%) of the dose is excreted in the urine, of which only a small proportion is unchanged (Table 6). The bioavailability of theophylline, after oral dosing, is close to unity (Hendeles et al., 1977). Theophylline is metabolised by *N*-demethylation and C-8-oxidation, with the latter route of elimination dominant (Table 6). The *N*-demethylation reactions are catalysed by human CYP1A2 in hepatic microsomes (Campbell et al., 1987), and in cell lines (Gu et al., 1992). Human CYP1A2 is also the high affinity, low capacity enzyme, catalysing the C-8-oxidation of theophylline, but with other isoforms (CYP2D6, 2E1 and 3A4) with low affinities and variable capacities also catalysing this reaction in vitro (Gu et al., 1992; Zhang and Kaminsky, 1995). The literature available supports the hypothesis that the primary routes of theophylline metabolism, at therapeutic concentrations, are predominantly mediated by the CYP1A2 isoenzyme.

Non-linear pharmacokinetics occurs at doses exceeding 10 mg/kg (Rovei et al., 1982), and only studies that used doses below this value were included in the current study. An analysis of the published data showed that

the mean pharmacokinetic clearance was 0.9 and 1.0 ml/min/kg after oral and intravenous dosing in 106 and 100 healthy adults, respectively (Table 7; see Dorne et al., 2001 for references).

3.4.2. Dog

After intravenous administration to dogs the majority (50–93%) of a dose is excreted in the urine (Rackley et al., 1991; Saunier et al., 1987), of which a large proportion is unchanged (Table 6). The two identified urinary metabolites are formed after C-8-oxidation and 1-*N*-demethylation (Table 6). There are no published data describing the involvement of specific isoforms of cytochrome P450 in the metabolism of theophylline in this species.

There are no data describing the non-linear kinetics of theophylline in the dog and the doses used for the current analysis ranged from 7 to 22 mg/kg. After an oral dose in four dogs the clearance value was 2.2 ml/min/kg (Munday et al., 1991), 2.4-fold greater than in humans (Table 7). After an intravenous dose the mean pharmacokinetic clearance in 25 dogs was 1.5 ml/min/kg (Clozel et al., 1981; Tse et al., 1981; Nosaka et al., 1986; Saunier et al., 1987; Brashear, 1989), 1.5 times the same value in humans (Table 7).

3.4.3. Rabbit

After intravenous administration to rabbits the majority of a single dose is excreted in the urine as metabolites (Table 6). Theophylline C-8-oxidation is a major route of elimination in the rabbit (Table 6) and this is catalysed by three isoforms of cytochrome P450 (CYP1A1, CYP1A2, and CYP2C3) in vitro, although more rapidly with CYP1A1 and CYP1A2 (McManus et al., 1988). Theophylline 1-*N*-demethylation is catalysed at similar rates by CYP1A1, CYP1A2 and CYP2C3, whereas 3-*N*-demethylation is more readily catalysed by CYP1A2 (McManus et al., 1988). These results suggest that CYP1A2 may catalyse most of the theophylline clearance in the rabbit in vivo.

Non-linear kinetics occur at doses exceeding 10 mg/kg (Gaspari and Bonati, 1990), and for the current analysis only those studies that used doses below this value were used. The mean oral clearance, in 18 rabbits, was 2.4 ml/min/kg (Kumar et al., 1991), 2.7 times faster than in humans (Table 7). After an intravenous dose the mean clearance in 63 rabbits was 1.5 ml/min/kg (Ng and Locock, 1979; May and Jarboe, 1981; Brashear et al., 1982; Wojcicki et al., 1996; Barakat et al., 1997), 1.5-fold greater than in humans (Table 7). The C_{max}/dose value, after oral dosing was 1311 (Kumar et al., 1991), 3.5-fold less than in humans (Table 7).

3.4.4. Rat

The absorption of theophylline from the gastrointestinal tract of the rat is extensive, with the majority (60–84%) of an oral dose of theophylline excreted in the urine (Lohmann and Meich, 1976; Williams et al., 1979; Teunissen et al., 1985; Salyers et al., 1994). A significant proportion of the parent compound is excreted unchanged (Table 6), which is dependent on the dose. The C-8-oxidation and 3-*N*-demethylation of theophylline are important routes of metabolism in the rat (Table 6). In vitro and in vivo metabolism studies support the view that both CYP1A2 and 2B1 can catalyse the C-8-oxidation of theophylline, with the former enzyme the more active (Lohmann and Meich, 1976; Williams et al., 1979; Bachmann et al., 1985; Salyers et al., 1994).

Saturation kinetics are observed at doses exceeding 10 mg/kg (Teunissen et al., 1985) and the studies used in this analysis ranged from 3 to 10 mg/kg. The mean plasma clearance of theophylline in 62 rats after an oral dose was 1.9 ml/min/kg (Groen et al., 1995), 2.1 times greater than in humans (Table 7). After an intravenous dose the mean clearance in 65 animals was 3.0 ml/min/kg (Teunissen et al., 1985; Arimori and Nakano, 1986; Larijani et al., 1986; Busby and Lesko, 1987; Zeruesenay et al., 1992; Angus et al., 1995; Poisson et al., 1996; Nakura et al., 1998), which is 3.0-times greater than the same parameter in humans (Table 7).

3.4.5. Mouse

Data after oral dosage in mice are not available. The majority of an intravenous dose is excreted in the urine of the mouse of which 6–20% represents the unchanged compound (Betlach and Tozer, 1980a). After a single intraperitoneal injection the partial clearance values for theophylline showed that both the C-8-oxidation and the 3-*N*-demethylation are equally important metabolic routes (Table 6).

Pretreatment with β-napthoflavone and 3-methylcholanthrene increased the rate of clearance of theophylline after a single intraperitoneal dose in some strains of "responsive" mice (C3H/HeJ, C57BL/6, BALB/c and A/J). In the responsive strains the total body clearance increased by a factor of 2- to 4-fold for β-naphthoflavone and 3-methylcholanthrene inducers, respectively and this was associated with an increased C-8-oxidation (Betlach and Tozer, 1980b). Konishi et al. (1995) demonstrated biphasic kinetics for C-8-oxidation and 3-*N*-demethylation reactions and showed that a high-affinity, low-capacity (CYP1A) and low-affinity, high-capacity (CYP2B/C) enzymes are involved in these reactions. Together, the data suggest that CYP1A2 is primarily involved in catalysing theophylline clearance in this species.

Linear pharmacokinetics have been shown to occur in the mouse at doses up to 100 mg/kg (Gaspari and Bonati, 1990). The clearance of theophylline in the mouse after intravenous dosage was 11.0 ml/min/kg (Betlach and Tozer, 1980a), 11 times greater than the same value in humans (Table 7).

4. Discussion

Considerable interspecies differences exist, not only in basic physiological processes, but also in the concentrations, amounts and nature of the enzymes contributing to the internal dose of a toxicant. However, an uncertainty factor of 4.0 (part of the 10-fold interspecies factor) has been considered sufficient to allow for interspecies differences in toxicokinetics, irrespective of the test species, the toxicant, or the route of elimination (WHO, 1994). Therefore, in the absence of specific kinetic data for a toxicant in the relevant test species and humans, the 4.0-fold default may generate an ADI value that is either an over- or under-estimation of the level that can be considered to be safe.

The purpose of the current work was to evaluate the validity, for CYP1A2, of the 4.0-fold factor for interspecies toxicokinetics in the test species commonly used for chronic toxicity studies (dog, rabbit, rat and mouse), using published data describing the metabolism and pharmacokinetics of human CYP1A2 substrates and to assess the usefulness of this information for generating species- and pathway-specific defaults for toxicokinetics. Although the validity of the interspecies kinetic factor has been analysed previously, this has generally been limited to only a few compounds, using a range of pharmacokinetic parameters, in one or two test species, and has not been related substantially to the metabolic fate of the chemical in humans (Kadry et al., 1997; Suh and Abdel-Rahman, 1997).

It is commonly believed that the differences in metabolism between species are likely to be a significant explanatory factor for interspecies variability in the internal dose of chemicals and therefore in the susceptibility of a test species to a toxicant (Voisin et al., 1990), although other factors that affect the internal dose such as absorption, bioavailability and excretion may also play a role (Renwick, 1993). The analysis of published data showed that the absorption and bioavailability of these structurally-related, probe substrates was high in humans and also in the test species considered, with the urine being the dominant route of excretion of the parent compound and its metabolites.

The primary metabolites of the CYP1A2 probe substrates were qualitatively similar between humans and all of the test species considered, although the rabbit was the only species which, when the data were available, consistently demonstrated qualitative, and quantitative similarities in metabolism to humans. Despite these interspecies differences in the routes of biotransformation, and the enzymes involved in this process, when the clearance parameter is considered, the rat and mouse fall outside the 4.0-fold default, with the former exceeding the default after oral administration only (Table 7). When the data were available the comparisons of pharmacokinetic clearance ratios, after oral and intravenous routes of administration, for most species, produced values that were not significantly different from each other (Table 7). The exception to this was the oral clearance value for caffeine in the rat, which generated a rat/human ratio which was 4.0-fold higher than after intravenous dosing, although this mean value was greatly influenced by data from one study (Bonati et al., 1984b). The clearance value for the other oral kinetic study in the rat was 4.9 ml/min/kg (Latini et al., 1980) which, by itself, would have generated an animal/human clearance ratio of 4.1.

When all compounds and all routes of administration were examined, the mean difference in the internal dose of the human CYP1A2 substrates for the different test species was:

Mouse (10.6) > Rat. (5.4) > Rabbit > (2.6) > Dog (1.6)

However, using compounds for which CYP1A2 was also shown to be responsible for catalysing the metabolism in the test species (a pathway-specific "categorical" default value), differences in the internal dose of the probe substrates for the mouse (caffeine and theophylline), rabbit (caffeine and theophylline) and the rat (theophylline) were:

Mouse (10.6) > Rabbit (3.0) > Rat (2.6)

The results of the analysis of the C_{max}/dose parameter were limited to the rabbit (theophylline only) since generally this value was not reported in the animal or human studies. Based on this limited database the ratio of human C_{max}/dose to rabbit C_{max}/dose was 3.5 (Table 7).

It is clear from this survey of the published literature that the quantitative metabolism of the CYP1A2 substrates in humans is generally different to that in the test species and the data do not always reflect a specific difference in CYP1A2 activity. The difference in biotransformation is more commonly a consequence of the preferential metabolism of a specific substrate by other enzyme(s) in the test species which in turn may, or may not be, more efficient than the human CYP1A2 enzyme. The extent of the differences in the internal dose for each test species, when the data were available, more commonly represented "animal CYP450-human CYP1A2" comparative values for toxicokinetics rather than pathway-specific "animal CYP1A2-human CYP1A2" "categorical" defaults. Therefore it would appear that irrespective of which specific dog oxidative enzyme(s) is (are) responsible for the metabolism of a toxicant, cleared by CYP1A2 in humans, it is likely that an ADI determination (using the current default of 4.0), derived from a chronic toxicity in this species, would generate a conservative exposure value for humans, which if replaced by a data-derived value may allow a larger ADI level. In contrast, assuming that the parent compound is the toxicant, an ADI which is derived

from a NOAEL in a chronic toxicity study in the mouse, using the default of 4.0, may be underestimating the likely human internal exposure, compared to that which would have been calculated using comparative toxicokinetic data for a chemical.

Interspecies differences were also shown for the dose at which non-linear kinetics occurs, and this would affect the magnitude of the difference in the internal dose between species. For theophylline and caffeine, all of the test species (for which data were available) showed saturable kinetics above 10 mg/kg except for the mouse, which demonstrated linear kinetics up to 100 mg/kg. This has important consequences in terms of the application of compound-specific, data-derived toxicokinetics since interspecies comparisons should be made using the NOAEL in animals, compared to pharmacokinetic values from humans administered the maximum anticipated intake. It is likely that the NOAEL value, for a human CYP1A2 substrate, will show non-linear pharmacokinetics in the rabbit and rat, which may not occur in the mouse at the equivalent dose. As the maximum anticipated intake in humans will most probably be a dose which is substantially lower than that in the test species, it is likely that humans will show linear pharmacokinetics. The toxicological sensitivity of each test species may therefore be party attributed to the saturation of enzymes responsible for the elimination of a toxicant. A test species which shows linear pharmacokinetics for a toxicant may metabolise it more rapidly resulting in lower internal exposure at the equivalent dose, and hence a higher NOAEL. These basic interspecies differences in biological processes involved in determining an internal dose, emphasise the need for more detailed characterisations of the pharmacokinetics and metabolism of specific toxicants in the most sensitive test species and humans. In terms of the generation of "categorical" defaults, it is clear that the basic information on P450 specificity is currently at a more advanced stage in humans, particularly in vitro, than in other species such as the dog and rabbit and indeed even the rat and mouse.

The default uncertainty factor to allow for interspecies differences in toxicokinetics, used in risk assessment, is not an accurate number, since the underlying data supporting this demonstrate wide variability. The current work has shown that the incorporation of all available scientific data into the regulatory framework, including that describing the "categorical processes", will ultimately reduce uncertainty in the risk assessment procedure. This work, in turn, demonstrates the importance of moving away from defaults by the use of compound-specific, data-derived uncertainty (or adjustment) factors, using the relevant test species, resulting in non-cancer risk assessments in which greater confidence can be placed.

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